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(54) Title: RAPID FATTY ACID DELIVERY

(57) **Abstract:** A method for rapidly supplying fatty acids to the bowel of a patient suffering from a gastrointestinal condition. The condition may be one in which gut microflora are reduced and/or modified. Such conditions include those in which rapid correction of gut microflora modification and/or reduction is required. Examples include diarrhoea, post operative surgery, gastrointestinal bacterial infections, antibiotic treatment, chemotherapy and radiotherapy treatments. The method includes the step of administering a fatty acid delivery agent orally. The fatty acid delivery agent is a fatty acid covalently bonded to a carrier molecule by a bond susceptible to microbial hydrolysis by bacterial hydrolases in the bowel to thereby release the fatty acid in the bowel and increase the level of the fatty acid. The increase in levels of fatty acid is rapid in relation to an increase in fatty acid levels due to fermentation of ingested carbohydrate so that at least one of the effects of the condition are ameliorated rapidly after administration.

RAPID FATTY ACID DELIVERY

FIELD OF THE INVENTION

This invention relates to methods for the rapid delivery of fatty acids to the gastrointestinal tract, and in particular to the large bowel, but also more generally to sites in the gastrointestinal tract where bacterial hydrolases are capable of cleaving fatty acids bonded to a carrier. The invention may also reside in formulations including a fatty acid delivery agent, and in methods of treating certain conditions.

BACKGROUND OF THE INVENTION

Gastrointestinal tract microflora are able to ferment dietary carbohydrates into fatty acids that provide energy for gut epithelium and facilitate the absorption of water and electrolytes. It is for this reason that it has long been considered that fatty acids in the large bowel provide a health benefit.

Short chain fatty acids (SCFA) in particular are formed in the large bowel of humans and other omnivorous species (eg dogs, pigs) and also in monogastric herbivores (eg horses, rabbits) through the bacterial fermentation of dietary components which have escaped digestion in the small intestine. In human adults, children and weaned infants, the principal substrates are undigested carbohydrates, notably oligosaccharides (OS), starch which has escaped digestion (resistant starch, RS) and non-starch polysaccharides (NSP, major components of dietary fibre).

Fermentation of carbohydrate yields three major acids – acetate, propionate and butyrate. One of the consequences of the production of the acids is a lowering of pH which produces a potential benefit through the inhibition of the growth of potentially pathogenic bacteria with a consequent reduction in the risk of enteritis. The three major acids have a number of common beneficial effects including a trophic action on the large bowel thus preventing colonic atrophy which may occur, for example, after gastrointestinal surgery. SCFA also stimulate muscular contraction and enhance the flow of blood through the colon by relaxing the vasculature. SCFA stimulate the absorption of fluid and this reduces the risk of diarrhoeal disease. Butyrate and, to a lesser extent, propionate act to maintain a normal population of colonocytes by

opposing the growth of and promoting apoptosis (programmed cell death) of malignant cells.

Delivery of SCFA to the bowel by oral administration is generally by fibre sources because SCFA are labile and are readily degraded or removed in the stomach and upper small intestine. Accordingly SCFA require protection in order for them to successfully be delivered to the bowel via oral administration.

The generation of SCFA from fibre sources is subject to considerable uncertainty due to differences in the balance of the bacterial species between individuals and also in the intakes of RS, NSP and OS. There is also the consideration that the distribution of SCFA along the colon is subject to differences in transit. SCFA production falls as food moves from the proximal to the distal colon due to substrate depletion. Concentrations fall in consequence of this and also the fact that the acids are absorbed into the mucosa. Thus, SCFA levels are high in the proximal colon but low in the distal colon.

Consideration of the human gastrointestinal tract shows that food normally reaches the large bowel some 3-5 hours after ingestion and fermentation starts at that time. This transit time can be reduced by the use of enteral feeding tubes. However, some further time may elapse before SCFA levels start to rise because it is generally thought that time is required for bowel microflora to induce an appropriate enzyme complement and / or to bulk up before they are able to produce physiologically effective levels of SCFA. In addition any rise in levels is likely to be non-specific especially where the microflora of the bowel needs to adapt to produce the SCFA.

Accordingly, a problem with oral methods of delivering fatty acids to the bowel is that there is a significant lag time between ingestion of material and an increase in fatty acid level in the bowel. However, it is a recognised maxim that following any trauma or adverse event the more rapid the remediation is started, the more rapid the recovery. Therefore in patients suffering from a condition in which gut microflora have been detrimentally modified or reduced, such as following gastrointestinal tract surgery, rapid delivery of SCFA to the bowel is required and it is necessary to resort

to alternative, more invasive methods of delivery such as direct delivery of fatty acid solutions via rectal enema.

There is therefore a need for an oral method of delivering the fatty acid so as to give a more rapid rise in levels in the bowel to more rapidly ameliorate the effects of a condition in which gut microflora have been detrimentally modified or reduced.

For the purpose of this specification the word "comprising" means "including but not limited to", and the word "comprise" has a corresponding meaning. Reference in this specification to a document is not to be taken as an admission that the disclosure therein constitutes common general knowledge in Australia.

OBJECT OF THE INVENTION

An object of this invention is to provide an oral method for the rapid delivery of at least one fatty acid to the gastrointestinal tract, or at least to provide the public with a useful choice.

SUMMARY OF THE INVENTION

The present inventors have previously shown that a fatty acid bonded to a carrier by a bond hydrolysable in the colon can give rise to an increased level of fatty acid in the colon. Hydrolysis of such bond releases the fatty acid and thereby provides for localised delivery of the specific fatty acid bonded thereto. WO 95/13801 which is incorporated herein in its entirety by reference discloses the use of a number of fatty acid delivery agents to deliver fatty acids to the large bowel. These fatty acid delivery agents comprise fatty acids covalently bonded to certain carriers by a bond that is cleaved by bacterial hydrolases in the large bowel.

The present invention arises from the finding that the accumulation of certain fatty acids delivered to the caeca of experimental animal is very rapid, and *in vitro* data shows that increases in levels of fatty acid can be detected at a time as short as between 30 minutes and 2 hours after administration.

Accordingly, in a first aspect the invention may be said to reside in a method for rapidly supplying fatty acids to the bowel of a patient suffering from a

gastrointestinal condition, the method including the step of administering a fatty acid delivery agent orally, said fatty acid delivery agent being a fatty acid covalently bonded to a carrier molecule by a bond susceptible to microbial hydrolysis by bacterial hydrolases in the bowel to thereby release the fatty acid in the bowel and increase the level of the fatty acid, said increase in levels being rapid in relation to an increase in fatty acid levels due to fermentation of ingested carbohydrate so that at least one of the effects of said condition are ameliorated rapidly after administration.

In one form the condition is an acute condition in which a rapid increase in levels of fatty acid is beneficial. For example, following gastrointestinal surgery it may be necessary to rapidly increase levels of fatty acids to promote growth and prevent atrophy of the gut wall. Accordingly levels of the fatty acid may need to be increased within about three hours of delivery of the fatty acid delivery agent to a part of the bowel. Using the method of the present invention and by administering the fatty acid delivery agent via enteral feeding tube, it may be possible to increase levels within one hour or less of delivery of the fatty acid delivery agent to a part of the bowel.

The gastrointestinal condition may be one in which gut microflora populations have been reduced and / or otherwise modified. By way of example, gastrointestinal surgery may result in resection of the tract and hence modification or reduction in the gut microflora population. Post operative recovery requires promotion of growth of microflora and prevention of atrophy. Rapid supply of fatty acids to the bowel may assist recovery by providing substrate and through trophic actions. However.invasive methods of delivery such as direct delivery of fatty acid solutions via rectal enema may lead to disturbance of the surgical site. The present method therefore provides for rapid delivery of fatty acid through oral administration and circumvents the need to use invasive methods.

An additional benefit of the present invention is that the method can not only be used to rapidly increase fatty acid levels in the bowel, but it may also be possible to obtain higher levels of one or more fatty acids in the bowel than is possible with other starch substrates. Thus for example, where butyrate is coupled by ester linkage to starch, it may be possible to selectively increase butyrate levels. This means that it is possible

that less of the delivery agent needs to be ingested to give a rise in levels equivalent to a rise as a result of ingestion of other carbohydrates.

The bond may be hydrolysable in the large bowel or the distal small bowel. Preferably the bond is hydrolysable in the large bowel.

The delivery might be to provide an increased level of the fatty acid in a part of the bowel within 48 hours of delivery of the fatty acid delivery agent to the bowel, preferably within 24 hours, more preferably within 3 hours and most preferably within 1 hour of delivery of the fatty acid delivery agent to the bowel.

The condition may be any condition in which the gut microflora is detrimentally affected. Examples of conditions in which rapid correction of gut microflora modification and/or reduction may be required include, but are not limited to, diarrhoea, post operative surgery, gastrointestinal bacterial infections, antibiotic treatment, chemotherapy and/or radiotherapy treatments.

In one form the fatty acid is selected from a list of fatty acids including short chain fatty acids, omega 3 fatty acids (including stearadonic acid), omega 6 fatty acids and conjugated fatty acids.

In a preferred form the fatty acid is one or more of the short chain fatty acids, which in the present context might be taken as having a carbon chain length of between 1 and 10. Preferably however the chain length is between 2 and 4, encompassing acetate, propionate and butyrate.

In one preferred form the carrier molecule to which the fatty acid is bonded is a carbohydrate. The carbohydrate may be selected from the list including pectins, gums and mucilages, cellulose, hemicelluloses, gums, inulin and oligosaccharides. In a particularly preferred form, the carrier is a starch. The starch may be digestible in the small intestine, but might in one form preferably be a resistant starch.

In one specific form of the invention the fatty acid is acetate and the carrier is a high amylose starch having greater than 80% amylose.

In another specific form of the invention the fatty acid is propionate and the carrier is a high amylose starch having greater than 80% amylose.

In another specific form of the invention the fatty acid is butyrate and the carrier is a high amylose starch having greater than 80% amylose.

Oral administration can take a number of forms, and can include ingestion of a tablet or capsule into which the fatty acid delivery agent is incorporated. A formulation of the fatty acid delivery agent may be in the form of a solid, liquid, gel or suspension. Preferably the formulation is a liquid because the transit time for a liquid in the gut is shorter than for a solid.

In one particularly preferred form the oral administration is by an enteral tube. This allows the fatty acid delivery agent to be delivered more directly to reduce transit time in the gut. For enteral feeding, the fatty acid delivery agent may be formulated in a liquid or suspension.

Preferably the formulation contains between 0.1 and 50 % by weight of the fatty acid delivery agent and most preferably between 0.5 and 20%.

In another specific form the formulation is a medicament in tablet, capsule, liquid, gel or suspension form. The formulation may include a pharmaceutically acceptable excipient which may be any suitable excipient and in one preferred form it is water. The fatty acid delivery agent may be provided as a solution or a suspension in water, or alternatively it may be provided as a dried powder that can be added to water to provide a solution, gel or suspension suitable for ingestion.

In a further specific form the formulation is an enteral formulation suitable for administration through a nasogastric tube.

In a second aspect, the invention might be said to reside in a method of orally delivering a fatty acid delivery agent in a physiologically acceptable medium to elevate the level of fatty acid in the bowel within a predetermined time period, the

fatty acid delivery agent being a fatty acid covalently bonded to a carrier molecule by a bond hydrolysable by bacterial hydrolases in the bowel to thereby release the fatty acid, the predetermined time period being prior to production of short chain fatty acids due to fermentation of carbohydrates by gut microflora.

It will be appreciated that there is generally a time lag between ingestion of carbohydrates and production of physiologically significant levels of short chain fatty acids in the bowel. The time lag is thought to be due, at least in part, to a period during which the gut microflora increase induction of enzymes of resident microflora and / or bulk up to a level at which they can produce physiologically significant levels of short chain fatty acids by fermentation of carbohydrates. The predetermined time period of the present invention therefore falls within the period of lag time between ingestion of substrate and production of physiologically effective levels of short chain fatty acids from carbohydrate.

The predetermined time period may be less than 48 hours, preferably less than 24 hours, more preferably less than 3 hours and most preferably less than 1 hour after delivery of the fatty acid delivery agent to the bowel.

In a third aspect the invention could be said to reside in a method for rapidly increasing electrolyte uptake in a part of the bowel of a patient suffering from an electrolyte imbalance, the method including the step of administering a fatty acid delivery agent orally, said fatty acid delivery agent being a fatty acid covalently bonded to a carrier molecule by a bond susceptible to microbial hydrolysis by microbial hydrolases in the gastrointestinal tract to thereby release the fatty acid and increase the level of the fatty acid in the bowel to thereby stimulate electrolyte uptake and reduce one or more of the effects of the electrolyte imbalance, said increase in levels being rapid in relation to an increase in fatty acid levels due to fermentation of ingested carbohydrate.

Electrolytes that may be affected by levels of short chain fatty acids include sodium and potassium.

The delivery might be to provide an increased level of the fatty acid in a part of the bowel within 48 hours of delivery of the fatty acid delivery agent to the bowel, preferably within 24 hours, more preferably within 3 hours and most preferably within 1 hour of delivery of the fatty acid delivery agent to the bowel.

In a fourth aspect the invention could be said to reside in a method of treating gastrointestinal bacterial infection by the rapid delivery of short chain fatty acids to the gastrointestinal tract, the method including the step of administering a fatty acid delivery agent orally, said fatty acid delivery agent being a short chain fatty acid covalently bonded to a carrier molecule by a bond susceptible to microbial hydrolysis by bacterial hydrolases in the gastrointestinal tract to thereby release the short chain fatty acid and increase the level of the short chain fatty acid in the gastrointestinal tract to stimulate electrolyte uptake and/or inhibit growth of bacterial pathogens, said increase in levels being rapid in relation to an increase in fatty acid levels due to fermentation of ingested carbohydrate.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a plot of the results of example 3 wherein the butyrate concentration of the caecum of a rat was determined at time points 2, 4 and 6 hours after ingestion of starch acetate ■ or a control fed starch 3401C ◆ ,

Figure 2 is a plot of the results of examples 3 and 4 wherein the concentration of acetate of the caecum of a rat was determined at time points 2, 4 and 6 hours after ingestion of starch acetate ■ or a control fed starch 3401C ◆ ,

Figure 3 is a plot of the results of examples 3 and 4 wherein the concentration of propionate of the caecum of a rat was determined at time points 2, 4 and 6 hours after ingestion of starch acetate ■ or a control fed starch 3401C ◆ ,

Figure 4 is a plot of the results of examples 3 and 4 wherein the concentration of butyrate of the caecum of a rat was determined at time points 2, 4 and 6 hours after ingestion of starch acetate ■ or a control fed starch 3401C ◆ ,

Figure 5 is a plot of the results of examples 3 and 4 wherein the concentration of total short chain fatty acids of the caecum of a rat was determined at time points 2, 4 and 6 hours after ingestion of starch acetate ■ or a control fed starch 3401C ◆ ,

Figure 6 is a plot of acetate (◆), propionate (■), butyrate (▲) and total SCFA (x) concentration vs time for in vitro fermentation of Hi-Maize™ high amylose maize starch. The SCFA concentrations are corrected for initial values (Hi-Maize-blank),

Figure 7 is a plot of acetate (◆), propionate (■), butyrate (▲) and total SCFA (x) concentration vs time for in vitro fermentation of cornstarch. The SCFA concentrations are corrected for initial values (Cornstarch-blank),

Figure 8 is a plot of acetate (◆), propionate (■), butyrate (▲) and total SCFA (x) concentration vs time for in vitro fermentation of acetylated starch (1% w/v). The SCFA concentrations are corrected for initial values (blank result has been subtracted), and

Figure 9 is a plot of acetate (◆), propionate (■), butyrate (▲) and total SCFA (x) concentration vs time for in vitro fermentation of butyrylated starch (1% w/v). The SCFA concentrations are corrected for initial values (blank result has been subtracted).

DETAILED DESCRIPTION OF THE EXEMPLIFIED EMBODIMENTS

THE FATTY ACID DELIVERY AGENT

The fatty acid delivery agent is either soluble in water or the lipid phase of the prepared formulation or alternatively can be rendered stable by an emulsifying agent such as by packaging into liposomes. Most preferably the fatty acid delivery agent is soluble in water for ease of administration.

The fatty acids are selected as being of benefit to the health of the individual human or animal, and preferably are selected from ones that give a short term benefit in terms of recovery or prevention of a visceral condition. The fatty acid might be one or more of the short chain fatty acids, which in the present context might be taken as having a carbon chain length of between 1 and 10. Preferably however the chain length is between 2 and 4, encompassing acetate, propionate and butyrate, from the literature these three SCFAs have the most evident health benefits. Alternatively a broader range of fatty acids are contemplated by this invention, which fatty acids play a role in benefits other than bowel health directly, and such fatty acids might be selected from the omega 3 fats (such as stearadonic acid, eicosapentaenoic acid, EPA and docosahexenoic acid DHA, linolenic acid), omega 6 fats (such as linoleic acid), and conjugated fatty acids (such as conjugated linoleic acid). These fats may be given as triacylglycerols or partial glycerides or as phospholipids bonded to the carrier.

One specific form of the invention relates to the delivery of short chain fatty acids.

The carrier can be varied greatly and might include natural dietary fibre or non-digestible oligosaccharides or other biological molecules, alternatively a synthetic polymer might be used as the carrier. The carrier might thus be contemplated as being a faecal bulking agent. The invention however contemplates that the carrier will be capable of being used as an energy source for normal large bowel microflora. Generally it is anticipated that the carrier will preferably be a carbohydrate so that on cleavage of the fatty acid from the carrier, the carrier can then be used, firstly as a means for increasing the microflora of the large bowel, and secondly can be metabolised by at least a proportion of the microflora to form SCFA, to further enhance health benefits to the large bowel. More preferably the carrier is a starch and most preferably a resistant starch.

The degree of substitution is also of relevance in so far as many carriers that might be contemplated such as for example hydrolysed carbohydrates would have a tendency to exert osmotic effects that might, for example, give rise to diarrhoea. The latter condition is predisposed to some extent already by the adoption of a radically different diet and the absence of SCFA which facilitates fluid absorption. Whereas

with suitable substitution the nature of a carrier molecule can be modified, so as to be a little more conducive to water retention by the large bowel. Additionally where the carrier is a natural carbohydrate such as a starch the substitution has a tendency to minimise gelatinisation, especially under heat treatment, thereby maintaining the resistance to digestion of the formulation by human enzymes in the small intestine after treatment for sterilisation. Additionally this will impact positively on the physical characteristics of the prepared formulation.

Examples of the bond between the fatty acid and the carrier are amide, ester or ether bonds.

The fatty acid delivery agent might be an acetylated resistant starch where the acetylation is made according to an aqueous method such as by the method of example 6.

The SCFA that are considered to be most beneficial in treatment or prevention of certain colonic disorders are those fatty acids with carbon chain lengths of 2, 3 and 4, namely acetate, propionate, and butyrate. However other SCFA may also have beneficial effects and therefore the term SCFA includes branched chain or substituted short chain fatty acids. There is doubt as to whether formate (C1) is of benefit in adults but may be of benefit in children (Bird *et al. Curr Issues Intest Microbiol* (2000) 1(1):25-37). SCFA of other lengths may also be beneficial so that the term SCFA is to be understood to include those fatty acids with a chain length in the range of between and including 1 to 6 carbons, and accordingly caproic and valerate and isovalerate are included in that description. It is also to be understood that fatty acids with longer carbon chain lengths may also be beneficial and may be covalently bonded to a carrier in a similar fashion. The fatty acids envisioned by this invention are all susceptible to breakdown before arriving at the colon, unless protected.

Other fatty acids that might also be contemplated by this invention might include omega-3 polyunsaturated fatty acids such as linolenic acid (18:3), eicosapentaenoic acid (20:5), docosahexaenoic acid (22:6), and stearadonic acid. The fatty acid delivery agent might include substitution by more than one fatty acid, or class of fatty acid.

The carrier to which the SCFA is bonded is preferably a carbohydrate, although other carriers may also be used. Using a carbohydrate has several advantages, largely because of the availability of carbohydrates in commercial quantities and because the effects of carbohydrates in the alimentary tract are relatively well understood. Some forms of carrier are undesirable, for example protein is undesirable because after fermentation of the protein by-products are formed that have an adverse effect on the colon.

Several forms of carbohydrate may be used as a carrier, these may include soluble non-starch polysaccharides, insoluble non-starch polysaccharides and oligosaccharides. The carbohydrates used may include but are not limited to pectins, gums and mucilages, celluloses, hemicelluloses, gums, inulin, oligosaccharides and glucans.

Any suitable source of pectin may be used and the following are illustrative of the types that might be used:- High, medium and low methoxylated pectins, high, medium and low gel strength pectins. The pectin may be derived from any number of sources which may include apples, oranges and lemons

Any suitable source of gums may be used and the following are illustrative of the types that could be used:- guar, xanthan, arabic, tragacanth, locust bean and psyllium. Modified and artificial gums may also act as a carrier.

The soluble non-starch polysaccharides may include inulin of varying chain lengths, pectin, chitin, β glucans, mucilages, agar, carageenans, alginates and similar. Most of these soluble fibres are fermentable for the largest part.

The insoluble non-starch polysaccharides may include cellulose (for example derived from oat hull, soybeans, cereal bran) and hemicellulose (mostly branched arabinoxylans or galactans, for example from cereals, potatoes or soybeans). Other celluloses may be used include, but are not limited to, microcrystalline and other chemically modified celluloses.

Oligosaccharides are understood to comprise any saccharide containing at least two and up to 10 monosaccharide units, whether of starch (α glucan) or non-starch type. Examples of oligosaccharides that might be used as carriers include fructo- and galacto-oligosaccharides such as hydrolysed inulin and levan (fructans), and short chain amylodextrins, malto dextrins and modifications and derivatives thereof.

The use of simple sugars such as glucose, fructose, galactose, sucrose and lactose, is limited because these may result in osmotic effects that lead to diarrhoea if administered at levels that are too high, however, it may be possible to use these in dilute solutions to effect an improvement in SCFA delivery.

One of the preferred forms of carrier is a starch because it can be fermented by microorganisms in the colon, and accordingly provides for extra nutrients for bacterial bulking in the colon, as well as separately providing a further source of SCFA additional to the SCFA linked to the carrier. Furthermore starch is readily available commercially.

The starch may be a starch that is digestible in the small intestine. Such digestible starch is protected to some extent from the degrading effects of α amylases in the small intestine by the SCFA bonded to it. The extent that the starch is protected will depend upon the degree of substitution, and if there is only a relatively low degree of substitution, then the starch will rapidly be degraded and there will be relatively good access by the low levels of esterases that exist in the upper alimentary tract to the ester bonds to cleave many of the SCFAs from the carrier thereby leading to ineffective delivery to the colon. It may therefore be advantageous to use a resistant starch that is already resistant to digestion in the small intestine, but that is digestible in the colon. This will maximise the delivery of starch, and probably SCFA.

The term starch is understood to include all forms of starch including modified starches, and the modification can be achieved physically, enzymically, by esterification, oxidation acid cleavage, and reaction with difunctional reagents, and includes those forms of starch that might be included in the classification RS1, RS2, RS3 and RS4. Starch can be derived from a great many sources, and may be derived, for example, be from native starches of wheat, potato, tapioca, maize, rice and oats.

The carrier may be a resistant starch which resists digestion because of its physical size, granular nature or starch type (e.g. high amylose maize). Such starch includes those found in potato, green banana and legumes such as peas and may occur additionally due to retrogradation following heat treatment causing granular disruption, hydration and subsequent reassociation in an enzymatic resistant form.

A high amylose starch is in one form a preferred carrier, because the acylation need not necessarily protect the carrier from digestion in the small intestine and because resistant starch carried through to the large bowel is known to be a particularly good substrate for colonic fermentation. Such a high amylose starch can be a quite high amylose starch having perhaps greater than 60% amylose or more preferably higher than 80% amylose. Examples of such starches are those available from Goodman Fielder, Melbourne, Australia under the name Hi Maize™.

It is to be understood that the carbohydrates listed may be modified, either singly or multiply though the use of:-

heat and/or moisture
physical treatment (e.g. ball milling)
enzymatic treatment (e.g. α or β amylase, pullulanase or the like)
chemical hydrolysis (wet or dry using liquid or gaseous reagents)
esterification (eg chemical with propylene oxide)
oxidation
cross bonding with difunctional reagents (e.g. sodium trimetaphosphate, phosphorous oxychloride)
carboxymethylation

or other forms of modification known to those practiced in the art. These can occur in aqueous and nonaqueous environments. This list of modifications is not intended to be exhaustive or limiting.

Where it is desired to deliver only one SCFA then a non-digestible carrier, i.e. one that is not degraded by bacterial enzymes of the colon is preferably used, leading to more accurate control over delivery of a single SCFA, and this may have beneficial effects on the treatment or prevention of certain disorders. The degree of substitution

coupled with the quantity of the agent ingested can be used to regulate the level of one or more SCFA delivered to the colon.

Other examples of fatty acid delivery agents can be determined by reference to WO 95/13801 which document is hereby incorporated by reference.

The daily dosage rate for a fatty acid delivery agent which takes the form of a starch substituted by 2 - 4 carbon length SCFA at a degree of substitution of about 0.25 could be in the range of 5 to 80 grams per day. This might be compared to a similar level of resistant starch requiring to be delivered at a rate in excess of 25 grams per day, to give the amount of SCFA required and demonstrated in W095/13801, and by Sheppach *et al.* (1992) *Gastroenterology*; 10: 51-56).

The daily dosage rate for a SCFA substituted onto a resistant starch such as a resistant maize starch at a degree of substitution of 0.25 may be in the range of about 5 to 80 grams per day although other dosage rates may be employed, and perhaps most preferably about 40 grams per day. It would be expected that similar dosages rates would be appropriate for other forms of the agent. However it may be beneficial to give a lesser amount. In the case of delivery of other fatty acids such as omega 3 fatty acids quite low levels may also give benefits.

The bond between the fatty acid and the carrier is one that can be cleaved by an agent in the bowel to give free fatty acid which can then be absorbed. It is to be understood that the cleavage can be either by a single enzyme, or may take a second step where that enzyme is present in or around the colon.

The bond between the fatty acid and the carrier is preferably an ester bond, because the capacity of the microbial flora of the large bowel to hydrolyse ester bonds is far greater than is the capacity of other portions of the alimentary tract to do so. Furthermore because hydroxyl groups are generally abundant amongst many carbohydrates there is a potential for a large range of densities of substitution and the ability to substitute is relatively easy.

Other forms of bonding may include amide bond to amino sugars, however such sugars are relatively rare in unmodified carbohydrates, and the rarity limits the extent of substitutions that might be made, or alternatively limits the usefulness to modified carbohydrates, some of which might have other specific advantages. Amide bonds are also relatively common as are enzymes capable of hydrolysing them, similarly ether linkages. Alternatively the link may be different where substituted fatty acids are used.

It might also be desired to add further components, for example specific vitamins, minerals, water- and fat-soluble anti-oxidants and additional pharmaceutical therapeutics or additives.

The degree of substitution can depend on the desired outcome, and degree of bulk or bacterial build-up that is desired. For example where a SCFA is bonded to a carbohydrate it is considered unlikely that the esterases will be able to access the ester bond between the sugar moiety and the SCFA moiety if more than one SCFA is present per carrier residue molecule. Furthermore it is likely that the surface characteristics of the carbohydrate will be modified to an extent that the carbohydrate will no longer be water soluble. In one form it is preferred that the degree of substitution be less than one per sugar moiety. However in another form it might be desired that the fatty acid delivery agent is soluble in the lipid phase of the nasogastric feed, and higher degrees of substitution might be acceptable than where the fatty acid delivery agent was to have been water soluble.

The term degree of substitution, will be understood, not to imply that each carrier molecule is evenly or equally substituted, but is to be taken as meaning an average degree of substitution. As in most substitution reactions, product molecules with a range of substitutions will result.

Where a digestible starch is used it is considered unlikely that any significant protection to cleavage will result if less than one SCFA is bonded for every twenty sugar molecules, accordingly in a preferred form of the first aspect of the invention the degree of substitution is selected from within the range of 0.05 to 1 SCFA per sugar moiety. Generally however for ease of synthesis and handling a range of

between about 0.1 and 0.5 is convenient. Other carbohydrates however are able still to be handled and solubilized where the degree of substitution is greater than one and therefore generally the degree of substitution is selected from the range of 0.05 to 2, and perhaps most conveniently is 0.25.

Additionally the benefit of the present formulation is that the level of a predetermined fatty acid can be increased in the large bowel. Thus, for example, a butyrylated starch could be added to specifically increase the level of butyrate in the large bowel.

ACETYLATED STARCH IN NASOGASTRIC FEED SOLUTIONS - EFFECTS ON LARGE BOWEL FERMENTATION

Methods

A measured dose of an enteral feeding solution including acetylated starch was administered by gavage to rats to mimic the ingestion of food whilst avoiding the mouth. Then, at intervals of approximately two hours, caecal digesta was collected and SCFA levels determined subsequently. Other changes in the intracolonic environment, relevant to bowel health, were also monitored.

For each study, adult rats (Sprague Dawley, 250-350 g) were maintained on a standard basal colony diet (Joint Stock Ration available from Ridley Agri Products, Murray Bridge, South Australia) before being allocated to two or three treatments and three time-point slaughter groups. After depriving rats of food overnight, they were gavage-fed (4ml) a slurry containing 1 g of either 3401C maize starch (Control, a high amylose starch available from Goodman Fielder, Melbourne, Australia) or acetylated starch (made according to Example 6). The gavage feed was through an FG8 tube presterilised (available from Indoplas Pty Ltd Sydney). The FG8 tube was 40cm in length with a 1mm internal diameter and a 2mm external diameter and positioned through the mouth and oesophagus to rest in the stomach. The solutions were forced through the FG8 tube using a syringe. At 2, 4 and 6 hours post-gavaging, rats were asphyxiated by CO_2 the abdominal cavity opened and the caecum excised. Caecal contents were expressed, weighed, diluted with a known quantity of internal SCFA standard (heptanoic (caproic) acid) and homogenised. After centrifugation (3000 rpm) supernatant pH was measured and then an aliquot stored

frozen to await analysis of SCFA. SCFA analysis was performed by the method described in Topping *et al.* (1993) *J Nutr.* 123:133-143.

Example 1 - Physical properties of various starch suspensions

All feeding solutions were prepared by suspending 1 g of designated starch in 4 ml of water. This was not sterilised.

- Acetylated Starch- thin slurry
- 3401C Control starch - thin slurry

Example 2 - Pilot study of gavage feeding with digestible starch and acetylated starch

This study was a pilot study using 2 female rats of about 400 g. The control starch 3401C was compared with acetylated starch produced by example 6. After overnight food deprivation, rats were dosed and killed two hours later. The stomach of each of the rats were empty of contents, product was visible in the small intestine.

Results

Table 1

Starch	Caecal contents (g)	pH
3401C	1.90	7.82
Acetylated starch	1.90	7.58

Table 2

Starch	Acetate	Propionate	Butyrate
3401C		Mmol	
	22.9	8.8	3.9
Acetylated starch	25.7	8.9	6.6

The measurement of the short chain fatty acids is a calculation of the total caecal content (mmol).

There is a numerically greater amount of butyrate present in the caecum of the rat fed Acetylated starch when compared to the rat fed 3401C control starch. There is no appreciable change in the amount of caecal content, pH of the caecum, or the levels of acetate or propionate.

Example 3 - Time trial of gavage feeding

This trial involved 6 male rats weighing about 330 g. The starches used were 3401C control starch or acetylated starch made in accordance with the method set out in example 6. Feed preparation of 1.25 g of starch were added to 5 ml water and used fresh. Each dosage was 4 ml.

Rats were dosed with various starches and killed 2, 4 or 6 hours later. The stomachs of each of the rats was empty of contents but products visible in small intestine. The contents of the caeca were processed as set out in the description of the methods.

Results

Table 3

Starch	Caecal contents (g)	pH
3401C - 2 h	2.22	7.62
3401C - 4 h	1.65	7.72
3401C - 6 h	2.55	7.27
Acetylated starch - 2 h	1.60	7.72
Acetylated starch - 4 h	1.80	6.97
Acetylated starch - 6 h	2.24	6.14

Table 4

Starch	Acetate	Propionate	Butyrate
		Mmol	
3401C - 2 h	21.1	6.7	4.7
3401C - 4 h	22.0	7.6	4.1
3401C - 6 h	31.4	10.9	6.5
Acetylated starch - 2 h	24.6	7.7	4.5
Acetylated starch- 4 h	30.5	8.0	6.4
Acetylated starch - 6 h	34.5	10.1	11.4

It can be seen that there is a numerical difference in the level of butryate found in the caeca of rats both after 4 hours and 6 hours after gavage feeding. Figure 1 is a plot of the caecal butyrate concentration.

Example 4 - Time course effect of acetylated starch

This trial involved 15 male rats weighing about 370 g. The starches used were 3401C control starch or acetylated starch made in accordance with the method set out in example 6. Feed preparation of 1.25 g of starch were added to 5 ml water and used fresh. Each dosage was 4 ml, a control with only water (n=1 per time point) was also included.

Rats were dosed with various starches and killed 4, 6 and 8 hours later. The stomach of each of the rats was empty of contents but product was visible in the small intestine. The contents of the caeca were processed as set out in the description of the methods.

Results
Table 5

Starch	Time	Acetate	Propionate	Butyrate	Total	Caecal Content	pH
Water Control							
	4 hr	9.1	4.1	0.8	14.0	2.58	7.52
	6 hr	22.9	8.1	1.1	32.1	2.38	7.44
	8 hr	21.1	7.3	0.9	29.3	1.25	7.48
3401C Starch							
	4 hr	8.5	4.8	0.8	14.1	1.6	7.63
	4 hr	16.2	4.4	2.9	23.5	1.5	7.83
	6 hr	6.4	3	0.5	9.9	1.42	7.64
	6 hr	13.9	6.9	1.1	21.9	1.91	7.40
	8 hr	31.5	12.2	1.2	44.9	2.64	7.22
	8 hr	22.0	8.2	1.0	31.2	1.54	7.44
Acetylated Starch							
	4 hr	51.6	11.0	13.9	76.5	3.47	6.46
	4 hr	23.6	6.9	9.7	40.2	2.53	6.43
	6 hr	40.8	13.6	14.8	69.2	2.76	6.36
	6 hr	17.5	12.2	15.8	45.5	4.85	6.40
	8 hr	30.3	9.4	12.3	52.0	2.36	6.35
	8 hr	43.6	16.1	13.3	73.0	2.94	6.45

Caecal ammonia was also measured in rats killed at 6 hours and there is a significant difference compared with the control starch 3401C. Control measured 10.1 mM as opposed to the acetylated starch which measured 4.1mM. Figures 2, 3, 4, and 5 are

composite figures using data from Examples 3 and 4, showing the calculated amount of caecal acetate, caecal propionate, caecal butyrate and total SCFA (combination of acetate, propionate and butyrate). Times 4 and 6 hours are combined and time 8 is omitted. These data show that in all cases there is an increase in the amount of the SCFA concerned. Using time pooled data it can be seen that all values are significant (see table 6 below).

Of interest is to note the lack of appreciable difference between the water controls and the starch control. There is considerable variation in the numerical values of each sample.

The data in the table below represents the pooled data from the 3401C control and the rats fed acetylated starch.

Table 6. Concentration of individual and total SCFA in the caecum of rats gavaged with acetylated starch (Example 4)

Dietary Group	Acetate	Propionate	Butyrate	Total
		mmol/L		
Control Starch	16.4	6.6	1.3	24.3
Acetylated Starch	34.6	11.5	13.3	59.4
P value	0.039	0.023	0.001	0.069

Values are least square means of 6 male rats per group. Caecal contents were collected at 4, 6, and 8 hour post gavaging. As there were no significant (P>0.05) time effects values were averaged across the three sampling time points.

Example 5 - Time course effect of acetylated starch

This trial involved 36 male rats weighing about 330 g. The starches used were 3401C control starch or acetylated starch made in accordance with the method set out in Example 6. Feed preparation of 1.25 g of starch were added to 5 ml water and used fresh. Each dosage was 4 ml, a control with only water (n=1) was also included.

Rats were dosed with various starches and killed 2, 4 and 6 hours later. The stomachs of each of the rats was empty of contents but products were visible in the small intestine. The contents of the caeca were processed as in example 2.

Table 7

Control Starch	Caecal Content (g)	pH	Time (hours)	Starch acetate	Caecal Content (g)	pH
1	1.42	7.10	2	1	1.91	7.51
2	1.27	7.29		2	3.01	7.25
3	0.83	7.50		3	0.56	7.20
4	0.42	6.97		4	2.26	7.12
5	0.40	7.53		5	1.89	7.25
6	1.12	7.23		6	1.51	6.85
Mean	0.91	7.27		Mean	1.86	7.20
SD	0.43	0.22		SD	0.81	0.21
7	2.36	7.30	4	7	0.45	6.27
8	1.12	7.08		8	1.22	6.82
9	1.70	7.27		9	1.48	6.63
10	2.78	7.07		10	1.34	6.94
11	2.95	6.74		11	2.99	6.60
12	0.14	7.26		12	1.85	6.12
Mean	1.84	7.12		Mean	1.56	6.56
SD	1.08	0.21		SD	0.84	0.32
13	1.13	6.89	6	13	2.64	5.93
14	0.32	7.03		14	1.70	5.91
15	1.73	7.18		15	0.52	5.78
16	0.66	7.06		16	2.54	6.00
17	1.13	7.30		17	2.72	6.01
18	2.33	6.63		18	3.16	6.36
Mean	1.22	7.02		Mean	2.21	6.00
SD	0.73	0.23		SD	0.96	0.20

Table 8 Caecal SCFA pool (mM)

Control Starch	Acetate	Propio-nate	Buty-rate	Total	Time (h)	Starch Acetate	Acetate	Propio-nate	Buty-rate	Total
1	48.5	13.1	5.6	67.1	2	1	22.8	11.6	6.9	41.3
2	24.0	11.1	4.8	39.8		2	19.5	14.0	6.5	40.0
3	4.2	3.1	1.9	9.1		3	15.7	4.7	3.0	23.4
4	12.6	3.7	2.0	18.3		4	70.3	23.1	12.8	106.3
5	10.9	3.6	1.5	16.0		5	43.4	15.0	8.5	66.8
6	16.0	6.5	2.1	24.6		6	46.5	15.9	9.1	71.5
Mean	19.4	6.8	3.0	29.2		Mean	36.4	14.1	7.8	58.2
7	68.2	23.0	12.5	103.7	4	7	5.8	1.5	1.3	8.7
8	33.0	9.5	4.7	47.1		8	24.2	5.0	5.3	34.5
9	13.6	6.7	2.9	23.1		9	45.1	8.5	7.9	61.6
10	75.2	29.4	14.7	119.2		10	25.6	8.1	4.1	37.8
11	63.2	18.2	23.6	105.0		11	66.5	19.1	20.3	106.0
12	2.9	1.0	0.7	4.6		12	53.6	13.5	13.0	80.1
Mean	42.7	14.6	9.9	67.1		Mean	36.8	9.3	8.7	54.8
13	31.6	8.6	5.5	45.7	6	13	59.1	16.3	16.9	92.3
14	10.0	2.5	2.5	15.0		14	35.9	12.4	8.6	56.8
15	38.9	13.0	7.3	59.2		15	16.8	3.7	2.7	23.2
16	20.2	5.9	3.2	29.3		16	33.5	8.1	7.1	48.8
17	30.5	8.0	4.4	42.8		17	109.5	31.6	19.1	160.2
18	51.8	15.3	11.6	78.7		18	119.1	30.8	30.3	180.2
Mean	30.5	8.9	5.8	45.1		Mean	62.3	17.2	14.1	93.6

Examination of data show that SCFA were highest at 6 hours in rats fed acetylated starch, but examination of Table 7 shows a continuous decline in pH. This suggests that SCFA generation and attendant change of pH was occurring before a significant difference in actual amount of SCFA was measured. The 4 hour values are out of line

with that observation and those of the earlier data and for that reason the 2 hour and 6 hour time points only were analysed.

Table 9. Caecal pools of individual and total SCFA in rats gavaged with acetylated starch in Example 5, 2 and 6 hour time point only

Dietary Group	Acetate	Propionate	Butyrate	Total
		mmol/L		
Control Starch	24.9	7.9	4.4	37.1
Acetylated Starch	49.3	15.6	11.0	75.9
P value	0.033	0.017	0.009	0.022

Values are least squares means of 12 male rats per group. Caecal contents collected at 2 and 6 hours post gavaging. Data for 4 hour collection excluded. As there were no significant (P>0.05) time effects vales were averaged across the two sampling time points.

It can thus be seen that there is a statistically significant increase in the amount of the individual SCFA present in the caeca of the rats fed with acetylated starch when compared with the caeca of rats fed control starch.

Discussion

It can be seen that the introduction of acetylated starch results in a significant increase in the amounts of SCFA in the caeca of these animals. These SCFA are known to have a significant effect on bowel health, and in particular butyrate is implicated in regeneration and repair of colonocytes.

An important aspect of these results is the extremely short time span over which the physiological increase in the amount of SCFA is elicited without prior adaptation of the animals. The changes occur in less than two hours, and time trial studies (*infra*) show an increase after 30 minutes. The significance of that is that there is then provided a means of very rapid delivery of SCFA to the bowel using an enteral feeding method. It is speculated that there is no need for the resident microflora to

significantly adapt to the new source of SCFA. This has implications for individuals who could benefit from such a rapid and effective delivery mode of SCFA.

In the animal studies the animals had not previously been fed the fatty acid delivery agent and in that sense were naive, suggesting that at least for that particular class of fatty acid delivery agent, or cleavage of at least one specific type of bond, no adaptation of the bowel microflora is required. This may contrast with the formation of fatty acids such as short chain fatty acids (SCFA) from fermentable fibres. Human faecal fermentation studies also show a rapid rise in detectable fatty acid release and suggest that adaptation of a naive microflora is not essential.

The finding that an orally fed fatty acid delivery agent can lead to rapid release has particular applications, and these are set out below.

Given the ubiquity of the esterases it is likely that the presence of a mix of bacteria in the large bowel will be able to cleave the SCFA from the carrier, and therefore individuals with microflora that might be compromised by reason of treatments such as antibiotics or chemotherapy will also be readily able to enable delivery of the SCFA. It is anticipated that not only will esterase links to acetate be readily cleaved but also to other SCFA, and indeed other fatty acids. Similarly other bonds readily cleavable by most bacteria will also result in a similarly quick release and these might include ether bonds and amide bonds.

It is thought that this rapid release of fatty acid is not simply a result of the enteral tube feeding, and in vitro studies (*infra*) confirm this. However it is possible that enteral feeding of the fatty acid delivery agent in liquid or suspended form may provide for a quicker deposit of the fatty acid delivery agent to the site of microbial enzyme action and therefore release.

This result has particular significance to treatment where rapid delivery is desirable. In instances where a patient undergoes surgery, for example a colonic resection, and requires optimal conditions for repair of the bowel, an enteral formulation for delivery of SCFA to the large bowel is highly desirable, other applications of this finding are set out further below.

The invention is also useful for the short term such as in enteral feed formulations used for example in intensive care units of hospitals, or indeed other delivery means such as in a beverage or dry formulations such as a biscuit, or tablet or in a capsule.

Example 6 - Preparation of acetylated high amylose maize starch

The following chemicals and materials were used to prepare the acetylated HA (High Amylose) maize starch:

Table 10 - Reagents

HA maize starch [Hi-maize™] (Goodman Fielder Australia).	350kg.
Water.	560L.
Acetic anhydride.	24.5L.
Hydrogen peroxide [100vol.]	1.47L.
Sodium hydroxide [0.65M].	As required.
Hydrochloric acid [10M].	As required.

The method employed to acetylate the HA maize starch utilised the following protocol;

1. Measurement of the required quantity of water into the reaction vessel.
2. Addition of the starch.
3. Adjustment of the pH of the slurry to pH 8.0 ± 0.1 using sodium hydroxide solution.
4. Addition of the hydrogen peroxide.
5. Agitation of the slurry for 45 mins.
6. Addition of acetic anhydride while simultaneously adding sodium hydroxide solution.

The pH was maintained in the range 8.0 to 8.5. The reaction was completed in less than 30 mins.

7. Permit the slurry to mix for 30 mins. The pH was maintained in the range 8.0 to 8.5.
8. The pH was adjusted to 5.0 to 6.5 with hydrochloric acid.

9. The starch was washed, dried and ground [to pass through a 212 micron screen].
10. The recovered starch had a degree of substitution of 0.25

SYNTHESIS AND IN VITRO FERMENTATION OF ACETYLATED, PROPIONYLATED AND BUTYRYLATED STARCH

Methods

Example 7 - Synthesis of Acetylated, Propionylated and Butyrylated Starch

Aqueous dispersion rather than dissolution in dimethyl sulphoxide was used to synthesise acylated starches. Slurries of gram quantities of 3401C in water were prepared and the particular acid anhydride along with base (NaOH) added drop-wise at a rate that ensured a pH of about 8 was maintained. The resultant mixture was stirred for several hours and the acylated material filtered, washed and air-dried. It is possible to produce an acylated starch with a DS of 0.2 in a single step.

Example 8 - In vitro fermentation of starch products

The in vitro fermentation procedure was based on the technique used by Edwards *et. al.* (1997) *J. Sci. Food Agric.* 1996 71 209-217.

A variety of different starches were evaluated. Initial fermentations used glucose and 3401C as substrate. Subsequent studies compared 3401C with acetylated, propionylated and butyrylated starch synthesised by the water method. Samples of butyrilated starch prepared by the aqueous and DMSO methods were also subjected to in vitro fermentation.

Frozen human faecal samples from several healthy individuals participating in another study were used to prepare faecal innocula. Faecal samples were thawed diluted 5-fold with degassed phosphate buffer, thoroughly mixed and then faecal debris removed by filtration. Tubes containing 100 mg of test starches were innoculated with faecal suspensions and incubated at 37°C. Tubes devoid of substrate served as a blank. Aliquots were withdrawn after 24 hours, vacuum distilled and short chain fatty acids measured by gas chromatography. Initial SCFA concentration in inoculum was also measured. Fermentations were replicated 6-fold.

Results

Example 9 – Evaluation of Acetylated, Propionylated and Butyrylated Starch

The two innocula (from two different volunteers) that were used had contrasting SCFA levels. SCFA production (test starch minus blank incubation values) was quite variable. Also, our values for SCFA production from starch are lower than the published values. This discrepancy may be due the use of frozen faeces to prepare innocula as published data are for derived using freshly voided faeces that were processed in a way to ensure their anaerobic status was maintained.

Acetylated, propionylated and butyrylated starch prepared by the aqueous method were fermented using inoculum from one subject. Acetate production was in similar amounts for all modified starches however, both butyrylated starch and propionylated starch fermentation increased butyrate and propionate levels, respectively (Table 11). Results of butyrylated starch fermentations prepared by the DMSO method, although variable, also consistently produced higher levels of butyrate compared to the unmodified starch (3401C) (Table12).

Table 11
SCFA production after 24 hour incubation of acetylated, propionylated or butyrylated starch with a singe source of human faecal inoculum

Inoculum	Acetate	Propionate mmol/L	Butyrate
3401C			
Human #5 b	17.0	0.3	0.3
Acetylated starch (aqueous method)			
Human #5 b	41.1	1.0	0.7
Butyrylated Starch (aqueous method)			
Human #5 b	38.8	0.9	8.0
Propionylated starch (aqueous method)			
Human #5 b	38.4	7.5	1.8

Table 12
SCFA production after 24-hour incubation of butyrylated starch with various sources of human faecal innocula

Inoculum	Acetate	Propionate mmol/L	Butyrate
3401C			
Human #3	3.0	0.4	0.0
Human #4 a	1.0	0.0	0.6
Human #4 b	8.4	0.4	0.1
Human #5 a	13.2	0.3	0.3
Butyrylated Starch (DMSO method)			
Human #3	2.6	0.5	8.5
Human #4 a	5.1	0.5	9.5
Human #4 b	11.9	2.1	3.0
Human #5 a	18.1	0.1	6.2

It can be seen therefore that accumulation of SCFA in the colon were manifest within a period of 24 hours.

INVESTIGATION OF THE MINIMUM TIME BEFORE SCFA ACCUMULATION

The objective of the study was to test the hypothesis that acetylated, propionylated and/or butyrylated starch is fermented rapidly by faecal microflora. Control starches and acetylated and butyrylated starches were continuously fermented under strict anaerobic conditions in a conventional *in vitro* batch fermentation system and the time-dependence of changes in short-chain fatty acid production assessed. Human faeces were used as mixed inocula and strict anaerobic conditions were maintained using nitrogen. Fermentation was stopped at designated intervals up to 24 h and samples of media subsequently analysed for pH and individual short-chain fatty acids. The time-course of changes in SCFA for the different substrates was determined. Acetylated and butyrylated starches were synthesised by the aqueous method used for a previous study.

Example 10 - Experimental Protocol

The fermentation system and procedure was similar to that used by Edwards et al (1996) In vitro method for the quantification of starch by human faecal bacteria. J. Sci. Food Agric. 1996 71 209-217.

The fermentation medium has been adapted from Goering and Van Soest. (Goering HK, Van Soest PJ. Forage Fibre Analysis (Apparatus, Reagents, Procedures and some Applications). Agricultural Handbook 379. US Dept of Agriculture, 1979.) It contained the following (per L of distilled water): 2.5g trypticase, 125ul micromineral solution, 250ml buffer solution, 250ml macromineral solution and 1.25ul resazurine solution 0.1% (w/v).

<u>Fermentation medium (per L distilled water):</u>

2.5 g trypticase
125 ul micromineral solution
250 ml buffer solution
250 ml macromineral solution
1.25 ml resazurine solution 0.1% (w/v)
Made up to 1 L using distilled water
33.5 ml of reducing solution added to 1 L of medium and autoclaved at 121°C for 15 min

<u>Micromineral solution</u>

132g $CaCl_2 . 2H_2O$
100g $MnCl_2 .4H_2O$
10g $CoCl_2 .6H_2O$
80g $FeCl_3 .6H_2O$

<u>Buffer solution</u>

4g $(NH_4)HCO_3$
35g $NaHCO_3$

<u>Macromineral Solution</u>

5.7g Na_2HPO_4

6.2g $KH_2 PO_4$
0.6g $MgSO_47H_2O$

<u>Reducing Solution (per L of distilled water)</u>
6.25g cysteine hydrochloride
6.25 g $Na_2S.9H_2O$
40ml NaOH 1M

Human faecal samples from two apparently healthy volunteers were used as inocula. Fresh samples were combined, thoroughly homogenised, and dispersed in pre-reduced buffered peptone water (20g/100 ml).

Vials containing test starches were inoculated with faecal suspensions and incubated at 37°C. Tubes devoid of substrate served as a blank. Initial SCFA concentration in inoculum was also measured.

100 mg of reference carbohydrates (Cornstarch (3401C) and Hi-maize™), and either 10 or 100 mg of test samples acetylated starch or 100 mg butyrylated starch, were weighed into 20 ml vials and 8 ml medium added. Substrates were hydrated at 4°C for 1 h. Then, 2 ml of faecal inoculum was added to each vial (2% w/v). Vials were flushed with nitrogen, placed on a mechanical shaker (Bioline Platform Rocker Model 4100) and then incubated at 37°C for 24 h. Quadruplicate determinations were performed for each substrate, blank and time point. Vials containing no added substrate (blank) were included in the protocol.

At 0, 0.5, 1, 2.5, 5, 6.5 and 22.5h, vials were removed from the incubator and aliquots of fermentation media taken for immediate measurement of pH and subsequent analysis of short chain fatty acids (2ml). Signs of gas production were also recorded. Samples taken for SCFA were diluted with internal standard and thoroughly mixed. These were then centrifuged at 3500 rpm at 10°C for 10 min and short chain fatty acids quantified by gas chromatography

Results

SCFA concentrations for Cornstarch and Hi-maize changed little for the first 6.5 h of fermentation. Between 6.5 and 22.5 h, total SCFA concentration increased relative to the initial (blank) concentration (Figs 6 and 7). This change was due largely to an increase in acetate and to a lesser extent propionate. Modest amounts of butyrate were produced as a result of fermentation of these substrates. At 22.5 hours, total concentrations of 31.1 mM and 19.8 mM, respectively, were achieved for acetate from cornstarch and HiMaize (this total includes those SCFA due to the inoculum itself).

However, for the various acylated starches (100mg; 1%w/v), an increase in SCFA was detected much earlier. For acetylated starch, an increase in acetate levels was observed after only one hour of incubation (Fig 8), whereas no other substrates showed an increase in acetate at this time point relative to the blank. Acetate concentration continued to increase throughout the remaining fermentation period. Concentration of acetate at 22.5 hours following incubation with the acetylated starch substrate, and after adjusting for the blank, was 5-fold and 1.5 fold higher than for Hi-maize and cornstarch substrates, respectively. A final total concentration of 39.3mM was achieved with acetylated starch (this total includes those SCFA due to those present in the inoculum itself). After 22.5 hours, propionate levels were approximately 2.5-fold and 61-fold higher, and butyrate concentrations were 4.2-fold and 2-fold higher, when compared to Hi-maize and cornstarch, respectively. Final total concentrations were 11.9 mM and 4.7 mM for propionate and butyrate, respectively (this total includes those SCFA due to the inoculum itself).

In the case of butyrylated starch, butyrate levels were beginning to elevate after 0.5 hours, were markedly increased after just 1 h and then steadily increased throughout the remaining incubation period (Fig 9). At one hour, after adjusting for the blank, butyrate concentrations were 3.0 mmol/L whereas all the other substrates showed no increase in butyrate relative to the blank at this time point. Butyrate concentration with the butyrylated starch substrate at 22.5 hours was raised 5-fold, 22-fold and 10-fold, when compared with substrates acetylated starch, Hi-maize and Cornstarch respectively. A final total butyrate concentration of 14.7 mM was achieved (this total includes those SCFA due to the inoculum itself). Acetate and propionate

concentrations were comparable to the final concentrations achieved with acetylated starch (100mg).

Acetylated starch was also tested at a lower concentration (10mg; 0.1%w/v). The results from this incubation series were similar to those obtained with 100mg Hi-maize (1%w/v). Changes in pH reflect the pattern of SCFA production for the various substrates (Tables 13-15).

Table 13

	Time (h)	pH	Gas Production	SCFA (mmol/L) Acetic	Propionic	Butyric	Total	% of total SCFA A	P	B
Blank	0	8.78	none	3.47	1.06	1.03	5.56	63.02	18.80	18.18
	0.5	8.76	none	3.15	0.79	0.80	4.73	66.57	16.55	16.88
	1	8.74	none	2.63	0.66	0.69	3.97	66.13	16.57	17.31
	2.5	8.72	none	2.45	0.00	0.64	3.09	79.11	0.00	20.89
	5	8.55	none	3.25	0.00	0.67	3.92	82.73	0.00	17.27
	6.5	8.37	none	4.91	0.22	0.73	5.86	84.12	3.37	12.51
	22.5	7.61	none	14.59	2.17	2.22	18.97	76.87	11.38	11.74
C/Starch	0	8.78	none	3.47	1.06	1.03	5.56	63.02	18.80	18.18
	0.5	8.91	none	1.85	0.00	0.51	2.36	78.26	0.00	21.74
	1	8.86	none	2.15	0.28	0.52	2.95	73.84	8.21	17.95
	2.5	8.84	none	2.22	0.00	0.63	2.86	77.94	0.00	22.06
	5	8.67	none	2.78	0.00	0.63	3.40	81.51	0.00	18.49
	6.5	8.43	none	4.02	0.00	0.47	4.49	90.29	0.00	9.71
	22.5	6.23	gas	31.07	2.33	3.53	36.92	84.13	6.30	9.57
Hi-maize	0	8.78	none	3.47	1.06	1.03	5.56	63.02	18.80	18.18
	0.5	8.85	none	2.11	0.32	0.43	2.86	76.59	8.91	14.49
	1	8.84	none	2.35	0.14	0.56	3.05	77.43	3.99	18.57
	2.5	8.78	none	2.16	0.00	0.57	2.73	78.98	0.00	21.02
	5	8.69	none	2.73	0.00	0.43	3.16	87.49	0.00	12.51
	6.5	8.47	none	4.22	0.00	0.53	4.75	88.85	0.00	11.15
	22.5	7.18	none	19.75	6.10	2.80	28.66	68.72	21.28	10.00

Values of 0.00 are given where the levels of SCFA were below the limits of detection of the gas chromatograph.

Table 14

	Time (h)	pH	Gas Production	SCFA (mmol/L) Acetic	Propionic	Butyric	Total	% of total SCFA A	P	B
Acetylated starch 10 0.1%w/v	0	8.78	none	3.47	1.06	1.03	5.56	63.02	18.80	18.18
	0.5	8.76	none	2.81	0.33	0.60	3.73	75.79	7.95	16.26
	1	8.67	none	2.41	0.43	0.56	3.39	71.60	11.50	16.90
	2.5	8.56	none	2.62	0.00	0.39	3.01	87.84	0.00	12.10
	5	8.67	none	3.01	0.00	0.49	3.50	86.07	0.00	13.93
	6.5	8.42	none	5.21	0.00	0.45	5.65	92.29	0.00	7.71
	22.5	7.41	none	20.40	5.20	2.51	28.10	72.57	18.50	8.93
Acetylated starch 100 1%w/v	0	8.78	none	3.47	1.06	1.03	5.56	63.02	18.80	18.18
	0.5	8.85	none	0.00	0.00	0.00	0.00			
	1	8.84	none	3.82	0.68	0.65	5.14	74.19	13.17	12.64
	2.5	8.81	none	4.13	0.00	0.53	4.66	88.40	0.00	11.60
	5	8.26	none	7.13	0.00	0.64	7.77	91.74	0.00	8.26
	6.5	7.80	none	9.92	0.38	0.66	10.96	90.40	3.38	6.22
	22.5	6.59	gas	39.34	11.90	4.67	55.90	70.52	21.43	8.05
Butyrylated starch 1%w/v	0	8.78	none	3.47	1.06	1.03	5.56	63.02	18.80	18.18
	0.5	8.70	none	1.17	0.00	1.51	2.69	43.91	0.00	56.09
	1	8.65	none	2.51	0.62	3.69	6.81	36.82	9.02	54.15
	2.5	8.57	none	2.36	0.00	5.13	7.49	31.50	0.00	68.50
	5	8.34	none	3.18	0.00	7.40	10.58	30.22	0.00	69.78
	6.5	8.06	none	5.59	0.00	6.70	12.29	45.46	0.00	54.54
	22.5	5.88	gas	42.63	9.21	14.74	66.58	64.02	13.83	22.15

Values of 0.00 are given where the levels of SCFA were below the limits of detection of the gas chromatograph.

Table 15

SCFA concentrations (mmol/L) at one and 22.5 hours, after adjusting for the blank value at these time points

	Acetate	Propionate	Butyrate
<u>One hour</u>			
(With blank subtracted)			
Corn-starch	16.48	0.16	1.31
Hi-Maize	5.16	3.93	0.58
Starplus A (10mg)	5.81	3.03	0.29
Starplus A (100mg)	24.75	9.73	2.45
Starplus B (100mg)	28.05	7.04	12.52
22.5 hours			
(With blank subtracted)			
Corn-starch	-0.48	-0.38	-0.17
Hi-Maize	-0.28	-0.52	-0.13
Acetylated starch (10mg)	-0.22	-0.23	-0.13
Acetylated starch (100mg)	1.19	0.02	-0.04
Butyrylated starch (100mg)	-0.12	-0.04	3.00

The study has demonstrated that SCFA production from acetylated and butyrilated starch fermentation by human faecal microflora occurs more rapidly than from conventional and high-amylose maize starches.

APPLICATIONS OF THE INVENTION

One of the major limitations of providing a source of SCFA for delivery enterally is that free fatty acids are readily degraded before reaching the colon. Furthermore in the case of naive recipient such as are often the case in patients requiring nasogastric enteral feed, the presence of a bacterial population capable of giving a high level of SCFA derived from degrading starch or other fermentable carbohydrate is not necessarily always present, whereas enzymes capable of cleaving the typical ether or ester bonds are far more pervasively present.

Thus where a hospitalised patient who for example requires surgery to address colon cancer, and who has had antibiotic treatment, the microflora will be considerably compromised.

The invention might include a method for recovery from a medical treatment, the treatment resulting in an injury to the large bowel, such as surgery or treatment of a bowel cancer such as by chemotherapy.

It is to be understood that this invention also has application to both animals as well as humans and accordingly the SCFA substituted carrier can be used also in the treatment of animal colonic disorders.

Example 11 - Pharmaceutical Formulations

Formulations containing fatty acid delivery agent can be prepared according to Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Co., Easton Pa. U.S.A., 1990.

The fatty acid delivery agent can be administered orally to a human or animal either by itself, or in pharmaceutical compositions where it is mixed with suitable carriers or excipient(s). In addition to the fatty acid delivery agent, formulations of the invention will preferably comprise pharmaceutically acceptable carriers and excipients, such as binding agents; fillers; lubricants; disintegrants; or wetting agents, as is known in the art.

Use of pharmaceutically acceptable carriers to formulate the fatty acid delivery agent for the practice of the invention into dosages suitable for oral administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the formulations of the present invention can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levitating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Formulations suitable for use in the present invention include those in which the active ingredients are contained in an effective amount to achieve its intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the disclosure provided herein.

The formulations of the present invention may also be in the form of enteral feeds. Enteral nutritional products are liquid compositions commonly understood to be supplied to and utilised in the gastrointestinal tracts of patients. The liquid portion of a liquid enteral nutritional product will generally be mainly water, but may also include lesser or minor amounts of one or more liquid non-aqueous substances such as lipids, e.g., vegetable oil or marine oil.

Feeding to the gastrointestinal tract is typically done by use of a nasogastric tube extending through a nasal passage and the esophagus and thence to the stomach, or by use of a feeding tube extending through the abdominal wall to the stomach or small intestine.

A health benefit is provided to the recipient when an ingredient that is, or is believed to be, nutritionally or pharmaceutically important to the patient, or is otherwise medically important.

A useful amount of a beneficial agent that is dispersible in the medium of the liquid enteral nutritional product is an amount or quantity that is "physiologically effective" and is demonstrably so or reasonably expected to be physiologically effective with respect to a patient, i.e., in producing a detectable beneficial effect on the patient on either a short term or long term basis when fed as part of a liquid enteral nutritional product, or, is "diagnostically detectable", and is detectable in diagnosing a condition or disease.

Amongst the nutrients that are most likely to be added to conventional enteral nutritional compositions according to this invention are nutrients, such as, glutamine, vitamins, arginine, fermentable dietary fibers, non-fermentable dietary fibres, enzymes such as lipases, combinations of amino acids, oligosaccharides such as fructo-saccharides, pyruvate precursors such as pyruvamide, or pyruvyl-amino acids,

such as, pyruvyl-glycine, pyruvyl-alanine, pyruvyl-leucine, pyruvyl-valine, pyruvyl-sarcosamine and their amides, esters and salts, structured lipids, d-cyroinositol, lactoferrin, marine oils, and acidulents such as ascorbic acid.

Example 12 - Gastrointestinal bacterial infections

Bacterial infections cause diarrhoea and adverse systemic effects through the release of toxins by the pathogen(s). This may be manifest as fluid loss and/or toxaemia. Acylated carbohydrates offer considerable potential benefit in providing SCFA to simulate electrolyte uptake and inhibiting the growth of the pathogens. This may be achieve with acylated carbohydrates as a food or beverage. The invention may be applied to human adults, children and infants and animals of domestic importance including dogs, pigs and horses.

This invention thus contemplates methods of treating gastrointestinal bacterial infection by the rapid delivery of short chain fatty acids, to the large bowel or the distal small bowel, such as in the case of bacterial overgrowth. Indeed this is significant particularly with certain toxins because the administration of certain antibiotics are contraindicated largely because they induce the expression of toxin. The delivery of the fatty acid delivery agent of this invention in particular where the fatty acid is SCFA may play a role in the rapid reduction of localised inflammation or even bacterial or otherwise induced ulceration, perhaps specifically by the delivery of elevated levels of butyrate. The invention may contemplate formulations comprising a fatty acid delivery agent and an anti-inflammatory composition, perhaps also bonded in similar manner to a similar carrier for delivery to the same part of the bowel.

Similarly where the diarrhoea concerned requires rehydration this invention contemplates formulations which include the provision of a fatty acid delivery agent in rehydration formulations such as are known, for example sold under the name Gastrolyte™ or like formulations.

Example 13 - Diarrhoea

Diarrhoea can occur for a variety of reasons (including pharmaceutical treatments) and is manifest as fluid loss. Acylated carbohydrates offer considerable potential benefit in providing SCFA rapidly and specifically to simulate electrolyte uptake.

This may be achieved with acylated carbohydrates as a food or beverage. The concept may be applied to human adults, children and infants and animals of domestic importance including dogs, pigs and horses.

Example 14 - Post-operative oral treatment

Gastrointestinal surgery is often accompanied by resection of the tract with a need to promote growth and prevent atrophy. The prompt supply of SCFA will assist in that process through trophic actions and the provision of substrate. In patients after polypectomy or removal of a malignancy there may be an additional benefit through the delivery of butyrate. This may be achieved with acylated carbohydrates as a food or beverage. The concept may be applied to human adults, children and infants and animals of domestic importance including dogs, pigs and horses. The supply of the short chain fatty acid may be as an adjunct to other wound healing promoters or anti-inflammatory agents. It may be within the ambit of this invention to provide for a suture impregnated with the fatty acid delivery agent or other depot of fatty acid delivery agent for localised slow release of the fatty acid adjacent to the wound.

Example 15 - Chemotherapy and/or Radiotherapy

Both of these treatments are used in the management of patients with malignancy. They can lead to disturbances in the large bowel bacterial flora and so to diminished SCFA supply. These patients may also suffer from loss of appetite or changes in food preference. Rapid and effective delivery of SCFA will assist in the maintenance of the large bowel and to the therapy if the tumour is of the gastrointestinal tract. This may be achieved with acylated carbohydrates as a food or beverage. The delivery might preferably be performed shortly after the treatment so that adverse effects might be alleviated. The invention may be applied to human adults, children and infants and animals of domestic importance including dogs, pigs and horses.

Example 16 - Sports Beverages and Foods

Prolonged exercise is associated with a loss of flow of blood to the splanchnic bed due to the demands of muscular tissue in the limbs. This leads to a build up of blood lactate and loss of glucose production. SCFA, supplied promptly and effectively from the gut, can increase blood flow and, if supplied as propionate, can assist in enhancing hepatic glucose synthesis. If taken before exercise SCFA may defer fatigue and if taken after exercise can assist in speeding the time to recovery. The concept may be applied to human athletes and sporting animals including dogs and horses. It

is anticipated that the invention may also encompass formulations of known sports beverages, which generally comprise high sugar levels, in particular glucose, and perhaps certain salts as well as a fatty acid delivery agent which preferably is a SCFA and more preferably propionate bonded to a carrier which preferably is a starch, but might also be a sugar preferably a simple sugar such as glucose.

In a food product such as a bread the fatty acid delivery agent, for example acylated starch, may be added as a 10% substitution for flour. An example formulation includes: flour (90 parts); acylated starch (10 parts); fat (2 parts); salt (2 parts); improver (1 part) and yeast (2.5 parts).

Example 17 - Diabetic Beverages and Foods

The equilibration of blood by the liver is a function of several factors including oxygenation and low O_2 delivery leads to higher blood glucose. Oxygenation is improved through greater blood flow which may be achieved through the effective delivery of SCFA to the bowel which will lower blood glucose. This may be applied to humans with Type I or Type II diabetes.

Various features of the invention have been particularly shown and described in connection with the exemplified embodiment of the invention, however, it must be understood that these particular arrangements merely illustrate and that the invention is not limited thereto and can include various modifications falling within the spirit and scope of the invention.

CLAIMS

1. A method for rapidly supplying fatty acids to the bowel of a patient suffering from a gastrointestinal condition, the method including the step of administering a fatty acid delivery agent orally, said fatty acid delivery agent being a fatty acid covalently bonded to a carrier molecule by a bond susceptible to microbial hydrolysis by bacterial hydrolases in the bowel to thereby release the fatty acid in the bowel and increase the level of the fatty acid, said increase in levels being rapid in relation to an increase in fatty acid levels due to fermentation of ingested carbohydrate so that at least one of the effects of said condition are ameliorated rapidly after administration.

2. A method as in claim 1 wherein the bond is hydrolysable in the large bowel.

3. A method as in claim 1 wherein the condition is an acute condition in which increased levels of fatty acid are beneficial and levels of the fatty acid need to be increased within three hours of delivery of the fatty acid delivery agent to a part of the bowel.

4. A method as in claim 1 wherein the increase in level of the fatty acid in part of the bowel is within 48 hours of delivery of the fatty acid delivery agent to the bowel.

5. A method as in claim 1 wherein the increase in level of the fatty acid in part of the bowel is within 24 hours of delivery of the fatty acid delivery agent to the bowel.

6. A method as in claim 1 wherein the increase in level of the fatty acid in part of the bowel is within 3 hours of delivery of the fatty acid delivery agent to the bowel.

7. A method as in claim 1 wherein the increase in level of the fatty acid in part of the bowel is within 2 hours of delivery of the fatty acid delivery agent to the bowel.

8. A method as in claim 1 wherein the increase in level of the fatty acid in part of the bowel is within 1 hour of delivery of the fatty acid delivery agent to the bowel.

9. A method as in claim 8 wherein oral administration is by ingestion of a tablet, capsule or liquid into which the fatty acid delivery agent is incorporated.

10. A method as in claim 8 wherein oral administration is via an enteral tube.

11. A method as in claim 10 wherein the fatty acid is selected from the list of fatty acids including short chain fatty acids, omega 3 fatty acids, omega 6 fatty acids and conjugated fatty acids.

12. A method as in claim 11 wherein the fatty acid is one or more of the short chain fatty acids having a carbon chain length of between 1 and 10.

13. A method as in claim 12 wherein the short chain fatty acid is selected from the list including acetate, propionate and butyrate.

14. A method as in claim 12 wherein the carrier molecule to which the fatty acid is bonded to is a carbohydrate.

15. A method as in claim 14 wherein the carrier is a resistant starch.

16. A method as in claim 15 wherein the carrier is a high amylose starch having greater than 80% amylose.

17. A method as in claim 15 wherein the concentration of fatty acid delivery agent is greater than 1 % w/v.

18. A method as in claim 15 wherein the concentration of fatty acid delivery agent is 5 to 30%w/v.

19. A method as in claim 18 wherein the fatty acid is acetate.

20. A method as in claim 18 wherein the fatty acid is propionate.

21. A method as in claim 18 wherein the fatty acid is butyrate.

22. A method as in claim 19 wherein the increase in levels of acetate in the bowel occurs between 0.5 and 1 hour after delivery of the fatty acid delivery agent to the bowel.

23. A method as in claim 20 wherein the increase in levels of butyrate in the bowel occurs between 0.5 and 1 hour after delivery of the fatty acid delivery agent to the bowel.

24. A method as in claim 18 wherein the condition is selected from the list including diarrhoea, post operative surgery, gastrointestinal bacterial infections, antibiotic treatment, chemotherapy and radiotherapy treatments.

25. A method of orally delivering a fatty delivery agent in a physiologically acceptable medium to elevate the level of fatty acid in the bowel within a predetermined time period, the fatty acid delivery agent being a fatty acid covalently bonded to a carrier molecule by a bond hydrolysable by bacterial hydrolases in the bowel to thereby release the fatty acid, the predetermined time period being prior to production of short chain fatty acids due to fermentation of carbohydrates by gut microflora.

26. A method as in claim 25 wherein the predetermined time period falls within the period of time between ingestion of substrate and production of physiologically effective levels of short chain fatty acids from carbohydrate.

27. A method as in claim 25 wherein the increase in level of the fatty acid in part of the bowel is within 48 hours of delivery of the fatty acid delivery agent to the bowel.

28. A method as in claim 25 wherein the increase in level of the fatty acid in part of the bowel is within 24 hours of delivery of the fatty acid delivery agent to the bowel.

29. A method as in claim 25 wherein the increase in level of the fatty acid in part of the bowel is within 3 hours of delivery of the fatty acid delivery agent to the bowel.

30. A method as in claim 25 wherein the increase in level of the fatty acid in part of the bowel is within 2 hours of delivery of the fatty acid delivery agent to the bowel.

31. A method as in claim 25 wherein the increase in level of the fatty acid in part of the bowel is within 1 hour of delivery of the fatty acid delivery agent to the bowel.

32. A method as in claim 31 wherein oral administration is by ingestion of a tablet, capsule or liquid into which the fatty acid delivery agent is incorporated.

33. A method as in claim 31 wherein oral administration is via an enteral tube.

34. A method as in claim 33 wherein the fatty acid is selected from the list of fatty acids including short chain fatty acids, omega 3 fatty acids, omega 6 fatty acids, stearadonic acid and conjugated fatty acids.

35. A method as in claim 34 wherein the fatty acid is one or more of the short chain fatty acids having a carbon chain length of between 1 and 10.

36. A method as in claim 35 wherein the short chain fatty acid is selected from the list including acetate, propionate and butyrate.

37. A method as in claim 35 wherein the carrier molecule to which the fatty acid is bonded to is a carbohydrate.

38. A method as in claim 37 wherein the carrier is a resistant starch.

39. A method as in claim 38 wherein the carrier is a high amylose starch having greater than 80% amylose.

40. A method as in claim 38 wherein the fatty acid is acetate.

41. A method as in claim 38 wherein the fatty acid is propionate.

42. A method as in claim 38 wherein the fatty acid is butyrate.

43. A method as in claim 40 wherein the increase in levels of acetate in the bowel occurs between 0.5 and 1 hour after delivery of the fatty acid delivery agent to the bowel.

44. A method as in claim 42 wherein the increase in levels of butyrate in the bowel occurs between 0.5 and 1 hour after delivery of the fatty acid delivery agent to the bowel.

45. A method for rapidly increasing electrolyte uptake in a part of the bowel of a patient suffering from an electrolyte imbalance, the method including the step of administering a fatty acid delivery agent orally, said fatty acid delivery agent being a fatty acid covalently bonded to a carrier molecule by a bond susceptible to microbial hydrolysis by microbial hydrolases in the gastrointestinal tract to thereby release the fatty acid and increase the level of the fatty acid in the bowel to thereby stimulate electrolyte uptake and reduce one or more of the effects of the electrolyte imbalance, said increase in levels being rapid in relation to an increase in fatty acid levels due to fermentation of ingested carbohydrate.

46. A method as in claim 45 wherein the increase in level of the fatty acid in part of the bowel is within 48 hours of delivery of the fatty acid delivery agent to the bowel.

47. A method as in claim 45 wherein the increase in level of the fatty acid in part of the bowel is within 24 hours of delivery of the fatty acid delivery agent to the bowel.

48. A method as in claim 45 wherein the increase in level of the fatty acid in part of the bowel is within 3 hours of delivery of the fatty acid delivery agent to the bowel.

49. A method as in claim 45 wherein the increase in level of the fatty acid in part of the bowel is within 2 hours of delivery of the fatty acid delivery agent to the bowel.

50. A method as in claim 45 wherein the increase in level of the fatty acid in part of the bowel is within 1 hour of delivery of the fatty acid delivery agent to the bowel.

51. A method as in claim 50 wherein oral administration is by ingestion of a tablet, capsule or liquid into which the fatty acid delivery agent is incorporated.

52. A method as in claim 50 wherein oral administration is via an enteral tube.

53. A method as in claim 52 wherein the fatty acid is selected from the list of fatty acids including short chain fatty acids, omega 3 fatty acids, omega 6 fatty acids, stearadonic acid and conjugated fatty acids.

54. A method as in claim 53 wherein the fatty acid is one or more of the short chain fatty acids having a carbon chain length of between 1 and 10.

55. A method as in claim 54 wherein the short chain fatty acid is selected from the list including acetate, propionate and butyrate.

56. A method as in claim 54 wherein the carrier molecule to which the fatty acid is bonded to is a carbohydrate.

57. A method as in claim 56 wherein the carrier is a resistant starch.

58. A method as in claim 57 wherein the carrier is a high amylose starch having greater than 80% amylose.

59. A method as in claim 56 wherein the fatty acid is acetate.

60. A method as in claim 56 wherein the fatty acid is propionate.

61. A method as in claim 56 wherein the fatty acid is butyrate.

62. A method as in claim 59 wherein the increase in levels of acetate in the bowel occurs between 0.5 and 1 hour after delivery of the fatty acid delivery agent to the bowel.

63. A method as in claim 61 wherein the increase in levels of butyrate in the bowel occurs between 0.5 and 1 hour after delivery of the fatty acid delivery agent to the bowel.

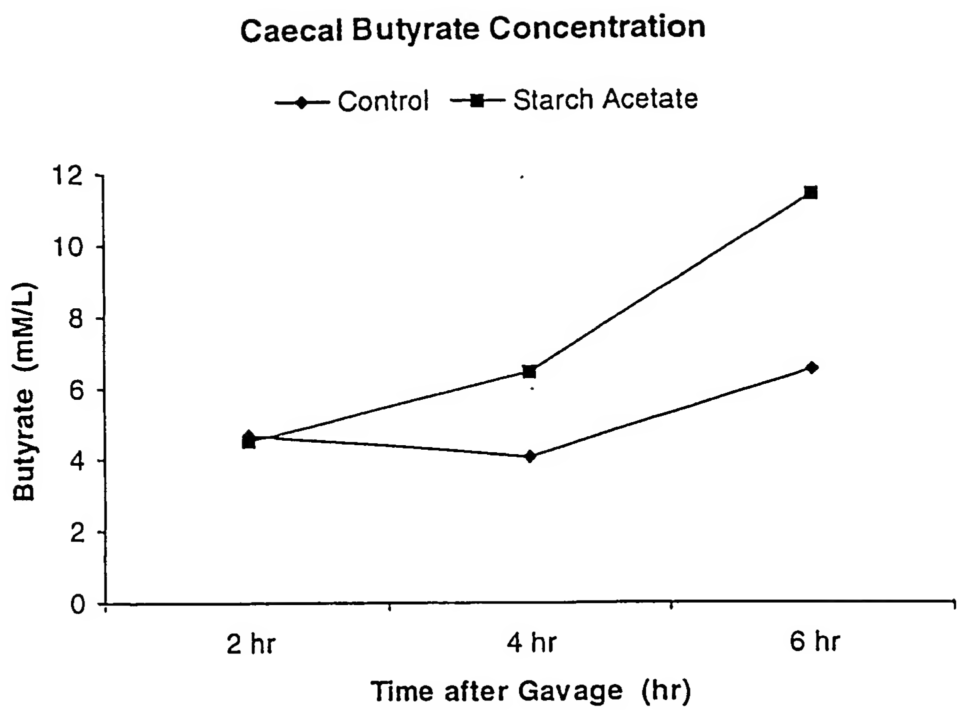

FIGURE 1

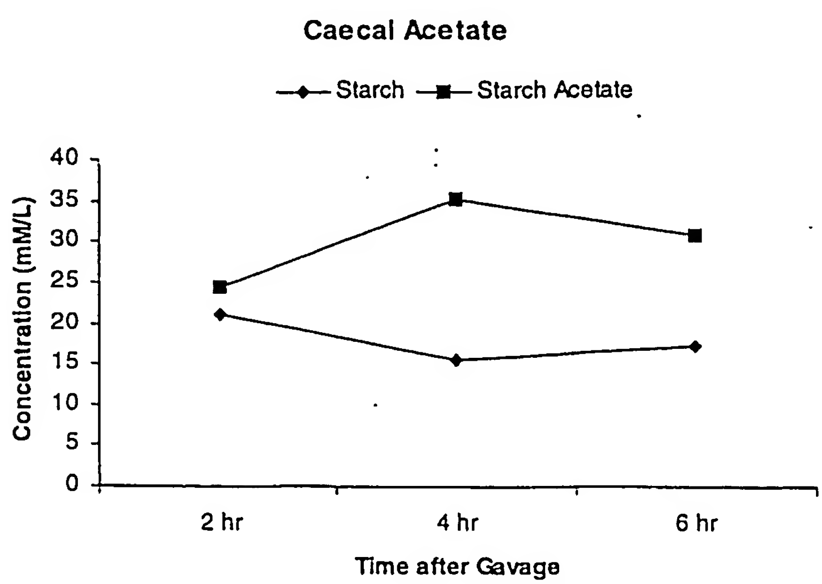

FIGURE 2

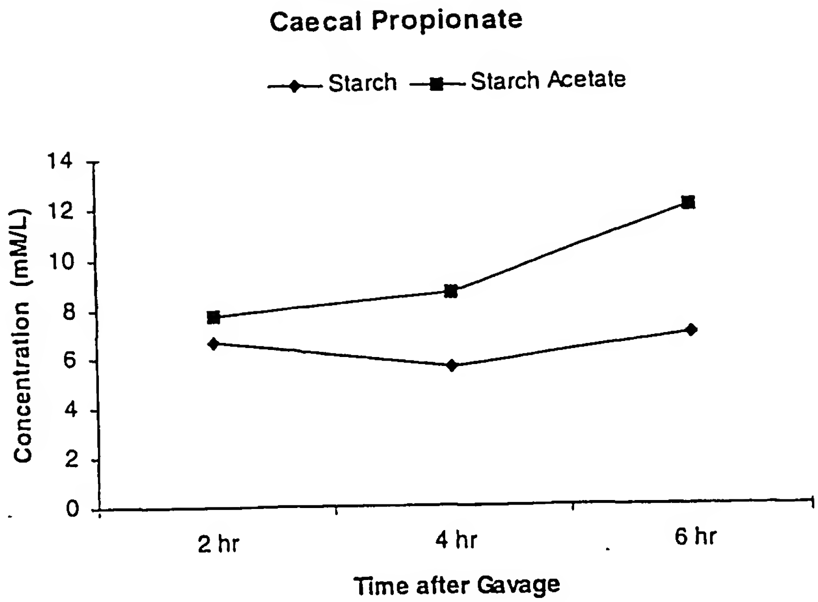

FIGURE 3

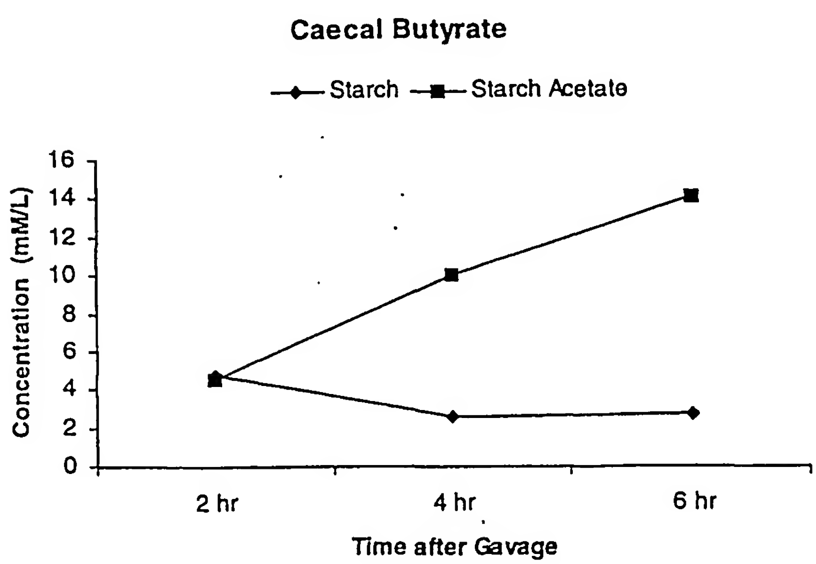

FIGURE 4

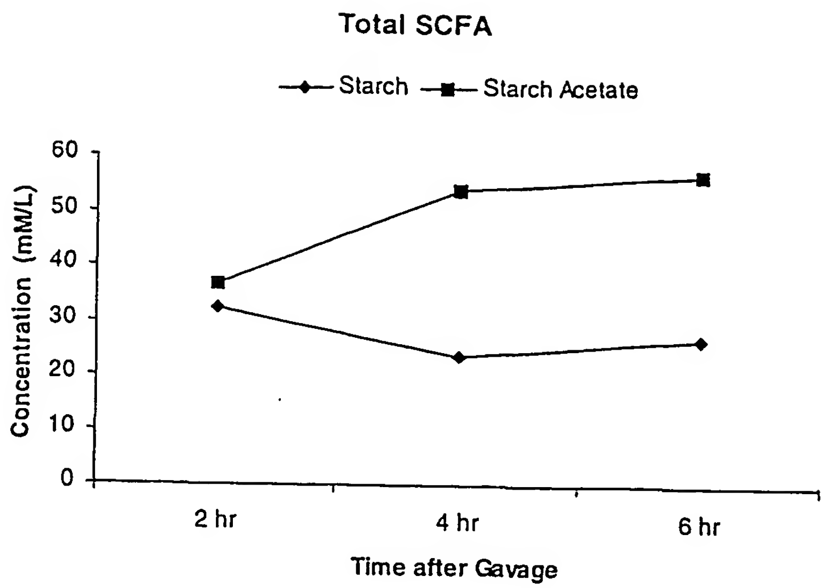
Total SCFA
Starch
Starch Acetate
60
50
40
30
20
10
0
Concentration (mM/L)
2 hr
4 hr
6 hr
Time after Gavage

FIGURE 5

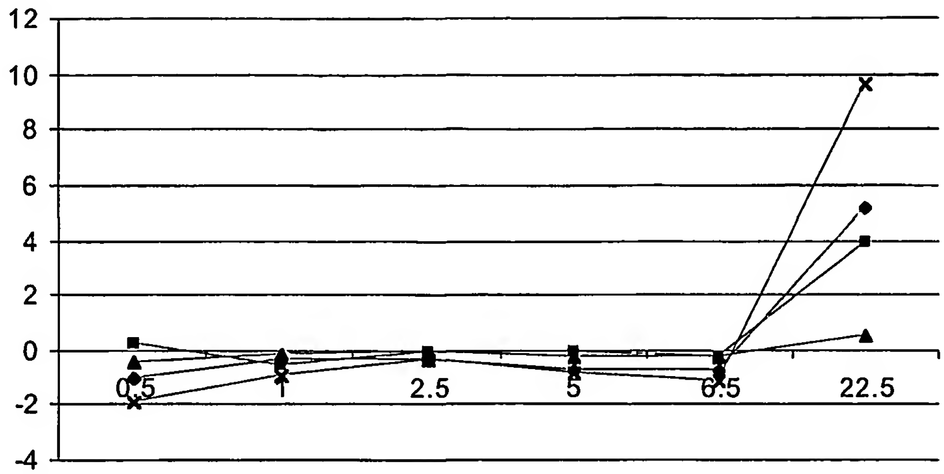

FIGURE 6

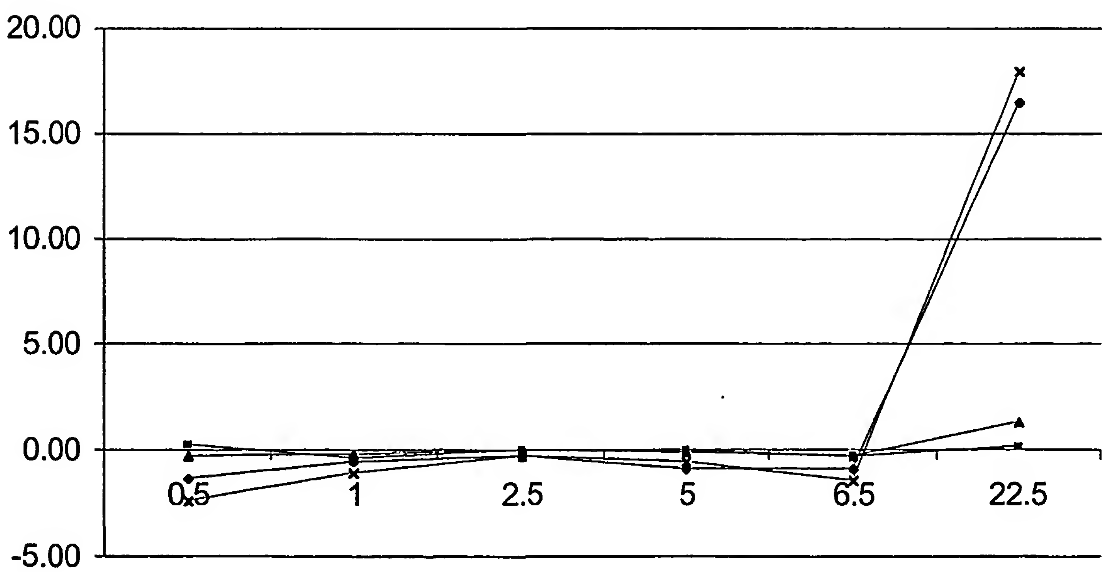

FIGURE 7

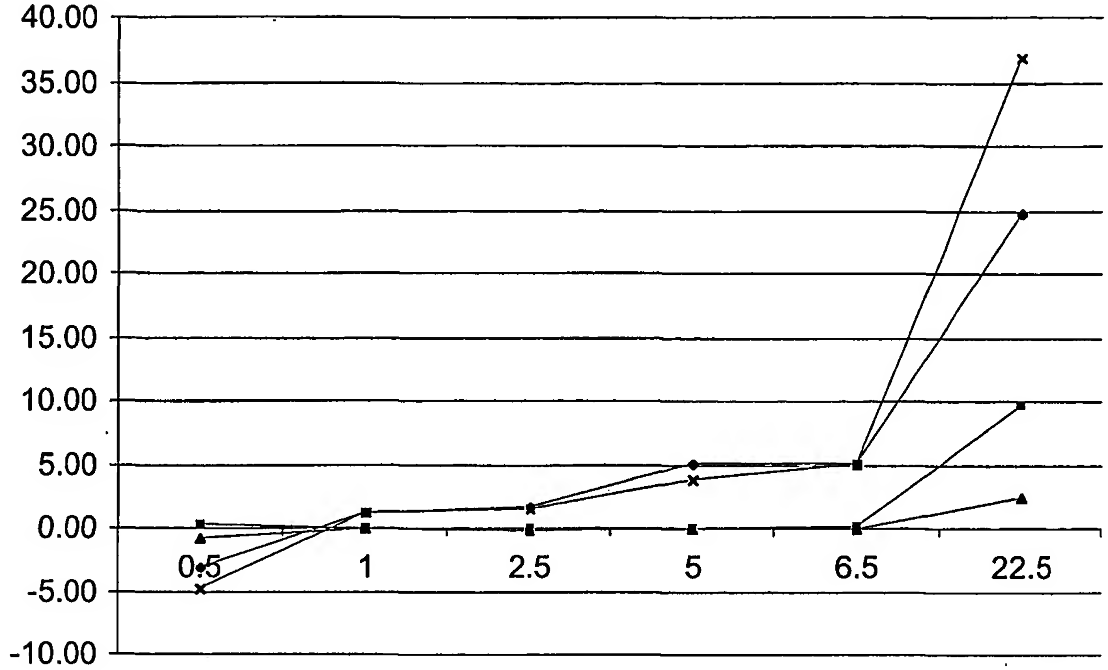

FIGURE 8

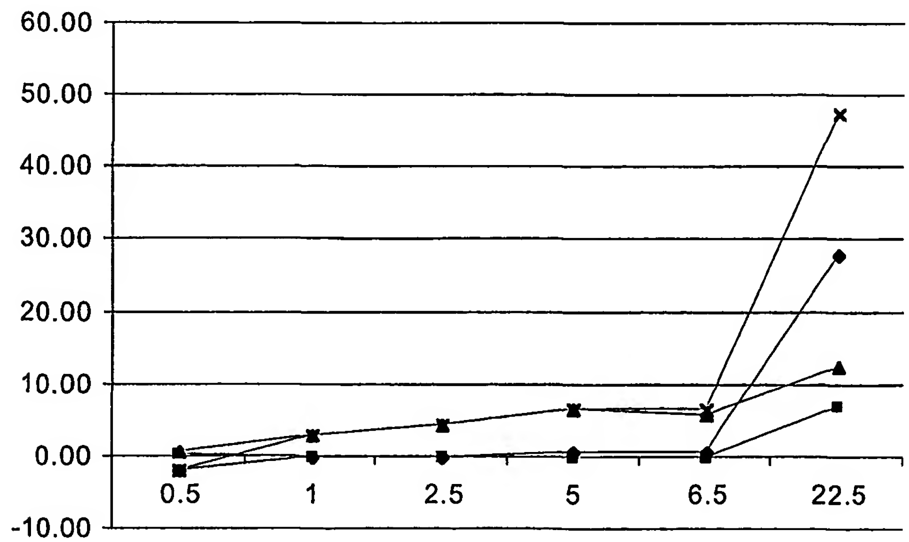

FIGURE 9

INTERNATIONAL SEARCH REPORT

International application No. **PCT/AU01/00766**

A.	**CLASSIFICATION OF SUBJECT MATTER**
Int Cl[7]:	A61K 31/19, 47/36, 47/38, A61P 3/12, 1/12, 1/00
	According to International Patent Classification (IPC) or to both national classification and IPC
B.	**FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)
A61K and key words as listed below

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT: Fatty acids, carbohydrate, starch and related terms, oral, bowel, gastrointest ( )
MEDLINE:

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95/13801A (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION), 26 May 1995 page 8 line 8 - page 9 line 32;page 11 lines 19-23; claims 1,24 - 26, 48 - 50	1-62
P,X	WO 01/02016A (COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANISATION), 11 January2001 page 4 lines 23-30, page 26 lines 21 - 28, claims 39-61	1-62
A	EP 451750 B (CLINTEC NUTRITION CO.), 16 October 1991 whole document	1-62

☐ Further documents are listed in the continuation of Box C

☒ See patent family annex

* Special categories of cited documents:

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Date of the actual completion of the international search 21 August 2001	Date of mailing of the international search report 31 AUGUST 2001
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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU01/00766

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
WO	9513801	AU	81368/94	CA	2176719	EP	730447
		US	5840860				
EP	451750	AU	74050/91	CA	2039980	JP	5306222
		US	5919822				
WO	200102016	AU	200055134				

END OF ANNEX